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Research Article

IN-VIVO ANTIOXIDANT EFFECT OF *ABROMA AUGUSTA* IN DIABETES INDUCED OXIDATIVE STRESS

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ABSTRACT

The present study was aimed to investigate the *In-vivo* antioxidant activity of roots of *Abroma augusta* in streptozotocin-nicotinamide induced Type-II diabetes. The extraction (Hot continuous extraction process) was carried out with solvents of different polarity. Commercially available Vit-E (100 mg/kg b.w.) was used as a standard drug. *In-vivo* antioxidant activity of plant extracts (250 mg/kg b.w.) was assessed by measuring SOD, CAT and LPO in the blood of Type-II diabetic animals. The results of the study revealed the significant effect on SOD, CAT and LPO level in animals treated with petroleum ether extract ($p < 0.0001$) followed by aqueous extract ($p < 0.001$) of *A. augusta* significantly as compared to diabetic control. The results of the study suggested the antioxidant activity of plant extract which prevents from oxidative stress and provide protection to vital tissues like liver, kidney, heart etc.

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INTRODUCTION:

Oxidative stress plays a significant role in the pathogenesis of diabetes. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins¹. Hyperglycaemia-induced glucose oxidation initiates membrane lipid peroxidation which is vital for the maintenance and integrity of cell function and initiates a non-enzymatic glycation of proteins, which in turn lead to enhanced production of ROS or result in decreased efficiency of inhibitory and scavenging system². Increase in levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of various complications of diabetes mellitus³.

A. augusta Linn (Sterculiaceae), also known as Ulatkambal (Bengali and Hindi), is a large spreading bushy shrub with fibrous barks and irritant hairs. It is widely distributed (native or collective) throughout the hotter parts of India, in U.P, Sikkim, Khasia Hills and Assam⁴. It is widely used in gynecological disorders and also used as abortifacient and anti-fertility agent. A study by Bhuyia *et al*⁵, reported the *in-vitro* antioxidant effect of leaves of *A. augusta*, whereby our study is

aimed to investigate the the *in-vivo* antioxidant effect of roots of *A. augusta* in diabetes induced oxidative stress.

MATERIAL AND METHODS:

Plant Material

The crude drugs of *Abroma augusta* Linn. (roots) were collected from the local herbal garden of Dehradun, Uttarakhand. The crude drug was authenticated at Forest Research Institute of India (FRI), Dehradun. The voucher specimen (No. 157029) of the plant was deposited in the Forest Research Institute herbarium. Soon after authentication collected parts of the plants were shade dried until they were free from moisture and were ground to coarse powder.

Chemical

Pyrogallol and hydrogen peroxide, phosphate buffer and Tris buffer were obtained from S.D. fine chemicals Ltd., India. Thiobarbituric acid (TBA), Trichloroacetic acid (TCA), 5, 5'-dithiobis (2- nitrobenzoic acid) (DTNB) were obtained from Sigma, USA. Vitamin E was procured from commercial sources.

Preparation of Extracts

The powdered plant material was extracted by Hot Continuous Extraction (Soxhletion) method. The extraction was carried out with solvents of different polarity in succession, starting with highly polar solvent

(petroleum ether, benzene, chloroform, acetone, and ethanol). Aqueous extract was prepared separately using chloroform water I.P. by maceration process ⁶.

Acute Toxicity Studies

Acute oral toxicity study for the test extracts of the plant was carried out using OECD/OECD guideline 425.

Limit Test at 2000 mg/kg

By performing Limit test at 2000 mg/kg of *A. augusta*, 250 mg/kg was found to be effective.

Antioxidant Activity

Assay Methods Used for Antioxidant Activity

Lipid Peroxidation (LPO)

To 2 ml, 5% suspension of separated RBC in 0.1 M phosphate buffered saline, 2ml of 28% trichloroacetic acid was added and centrifuged. 1ml of 1% thiobarbituric acid was added to 4 ml of supernatant, then heated in boiling water for 60 min and cooled immediately. The absorbance was measured at 532 nm by spectrophotometric method. On the basis of the molar extinction coefficient of malonaldehyde (MDA) (1.56×10^5) lipid peroxidation was calculated, and expressed in terms of nanomoles of MDA/g Hb.

Following formula was used to calculate LPO:

$$A = abc$$

A = Absorbance, a = Extinction coefficient, b = path length, c = Concentration

Superoxide Dismutase (SOD) It was estimated in the erythrocyte lysate prepared from the 5% RBC suspension. To 50 μ l of lysate, 75mM of Tris-HCl buffer (pH 8.2), 30mM EDTA and 2mM of pyrogallol were added. Absorbance was recorded spectrophotometrically at 420 nm for 3 min. One unit of enzyme activity is 50% inhibition of the rate of autooxidation of pyrogallol as determined by change in absorbance/min at 420 nm. The activity of SOD is expressed as units/mg protein.

Following formula was used to calculate SOD:

$$\% \text{ inhibition} = \frac{1 - (\Delta_{\text{blank}} - \Delta_{\text{sample}}) \times 100}{\Delta_{\text{blank}}}$$

Catalase (CAT)

Catalase activity was determined in erythrocyte lysate. 50 μ l of the lysate was added to a cuvette containing 2ml of phosphate buffer (pH 7) and 1ml of 30mM H₂O₂ (hydrogen peroxide). Catalase activity was measured at

240 nm for 1 min using spectrophotometer. The catalase activity was determined on the basis of molar extinction coefficient of H₂O₂ (43.6 M cm^{-1}). One unit of activity is equal to one millimoles of H₂O₂ degraded per minute. It is expressed as units per milligram of protein.

Following formula was used to calculate CAT:

$$A = abc$$

A = Absorbance, a = Extinction coefficient, b = path length, c = Concentration

Experimental Protocol for Antioxidant Activity

Induction of Type-II Diabetes

Diabetes was induced as per the method described by Ananda et al with slight modification (streptozotocin-nicotinamide induced Type-II diabetes). Animals showing fasting blood glucose higher than 250 mg/dl were considered as diabetic and used for the further study. Nine groups of animal having six rats in each group were used and all groups of animal received treatment for 7 days. **Group-1:** Normal control, **Group-2:** Diabetic control, **Group-3:** Diabetic animal+Vitamin E (standard drug) **Group-4:** Diabetic animal+Petroleum ether extract of *A. augusta*, **Group-5:** Diabetic animal+Benzene extract of *A. augusta*, **Group-6:** Diabetic animal+Chloroform extract of *A. augusta*, **Group-7:** Diabetic animal+Acetone extract of *A. augusta*, **Group-8:** Diabetic animal+Ethanol extract of *A. augusta*, **Group-9:** Diabetic animal+Aqueous extract of *A. augusta*.

Collection of Blood Sample and Evaluation of Antioxidant Activity

Blood sample was withdrawn on 7th day of the study from the retro-orbital plexus. After collecting the blood sample, above mentioned assay methods were used for evaluation of antioxidant activity of various extract of *A. augusta*.

Statistical Analysis: All the results were expressed as the mean \pm Standard error mean (SEM). Data was analyzed by using two way ANOVA followed by tukey's multiple comparison as post-hoc test. The limit of statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION:

Effect of *A. augusta* in Diabetes Induced Oxidative Stress: In the present investigation various extracts of *A. augusta* were screened for antioxidant activity. *In-vivo* antioxidant activity was assessed by measuring SOD, CAT and LPO in the blood of diabetic animals (**Table-1**).

Table 1: Effect of *A. augusta* on Oxidative Stress in Diabetic Rats

S. No.	Groups	Superoxide Dismutase (Units/mg protein)	Catalase (Units/mg protein)	Lipid Peroxidation (nmMDA/g Hb)
1	Normal	47.26 \pm 7.56	297.20 \pm 6.56	84.00 \pm 4.34
2	Dia. Control	16.19 \pm 4.35 ^{a*}	189.50 \pm 4.67 ^{a*}	189.5 \pm 5.89 ^{a*}
3	Dia. control + Vit. E	45.97 \pm 2.14 ^{b*}	301.0 \pm 1.98 ^{b*}	96.05 \pm 2.89 ^{a†b*}
4	Dia. control + Pet.ether extract	37.01 \pm 3.56 ^{a†b*}	201.26 \pm 5.56 ^{a* b*}	134.53 \pm 7.35 ^{a* b* c*}

5	Dia. control +Benzene extract	18.13± 3.67 ^{a*c*}	161.17±5.70 ^{a*c*}	189.82±4.79 ^{a*c*}
6	Dia. control + Chloroform extract	17.41 ± 4.98 ^{a*c*}	188.45±3.44 ^{a*c*}	185.41±3.68 ^{a*c*}
7	Dia. control + Acetone extract	19.14±9.12 ^{a*c*}	188.22±7.27 ^{a*c*}	182.36±9.45 ^{a*c*}
8	Dia.control + Ethanol extract	21.27±9.67 ^{a*c*}	187.47±2.45 ^{a*c*}	186.91±4.75 ^{a*c*}
9	Dia. control + Aqueous extract	29.80± 3.76 ^{a*b#c*}	190.41±2.87 ^{a*b†c*}	174.74±7.56 ^{a*b†c*}

a= Vs Normal control, b=Vs. Diabetic control, c=Vs. Active control; * = p<0.0001, † = p< 0.001, ‡ = p< 0.01, § = p<0.05

Effect on SOD and CAT level

SOD and CAT are two major antioxidant defense system of the body which protect the cell membrane and other cellular constituents against oxidative damage by free radical species and plays an important role in protecting the cell against the potentially deleterious effect of reactive oxygen species. In diabetic animals, serum concentration of SOD and CAT reduces, which may results in number of deleterious effects due to accumulation of superoxide radicals ($O_2^{\cdot-}$) and hydrogen peroxide. SOD and CAT level came close to normal level in animals treated with petroleum ether extract and aqueous extract of *A. augusta* in diabetic animals significantly as compared to diabetic control. The positive effect of extracts on antioxidant enzyme level clearly indicates the free radical scavenging activity of *A. augusta*, which could exert a beneficial effect against pathological changes caused by free radicals.

Effect on LPO: Increased free radical generation induces Lipid Peroxidation, refers to oxidative

degeneration of lipids that impairs cell membrane functions resulting into cell damage and leading to severe diseases. The most significant reduction on LPO was observed with petroleum ether extract followed by aqueous extract of *A. augusta* as shown in Table-1 suggests the antioxidant activity of plant extract which prevents from oxidative stress and provide protection to vital tissues like liver, kidney, heart etc.

CONCLUSION:

The results of the present study concluded that the petroleum ether extract of *A. augusta* possesses potent antioxidant and lipid peroxidation activity and can be employed in protecting tissue from the oxidative stress, which may be responsible for its hypoglycemic property. Further studies to isolate the active components of *A. augusta* are needed to explore the research on abovementioned plant usage in different oxidative stress induced diseases.

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